Synthesis and Degradation of pH-Sensitive Linear Poly(amidoamine)s

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Received October 6, 2006; Revised Manuscript Received December 7, 2006

ABSTRACT: The design and synthesis of a family of cationic, pH-sensitive poly(amidoamine)s are reported. Acetal or ketal linkages were incorporated into the backbone of the polymers to provide for degradation into low molecular weight hydrophilic compounds upon lowering of the pH. All of the polymers demonstrated a pH-dependent degradation profile with a very significant increase in hydrolysis rate as the pH was lowered from 7.4 to the pH value of 5.0 commonly found in lysosomes. The hydrolysis half-life of the poly(amidoamine)s varied from 0.03 to 81.5 days at pH 5.0 and from 6.0 to 161 days at pH 7.4, depending upon the structure of the components used to prepare each of the acid-degradable polymers. This data suggests that these new pH-sensitive hydrophilic poly(amidoamine)s and analogous structures may prove useful in the field of polymer therapeutics and drug delivery.

Introduction

Polymers are used as scaffolds for drug delivery systems due to their ability to enhance the properties of their therapeutic cargo. For example, polymers such as *N*-(2-hydroxypropyl)-methacrylamide and polyester dendrimers have been used to improve the solubility and pharmacokinetics of anticancer drugs.[1,2] Additionally, encapsulation of protein and plasmid DNA in partially degradable polyacrylamide protected the biotherapeutics from degradation in vivo and facilitated cytoplasmic delivery.[3,4]

Cationic polymers are a leading class of materials for drug delivery systems because of their diversity in terms of molecular weight, architecture, chemical structure, and their ability to bind biotherapeutics through ionic interactions.[5−7] Natural cationic polysaccharides, such as chitosan,[8] and synthetic polycations, such as poly(ethylene imine),[9−11] poly(L-lysine),[12−14] and poly(amidoamine)s,[15,16] have been used for delivery of protein, nucleic acids, and drug molecules. Currently available cationic polymers are nondegradable or contain linkages, such as amides, that degrade slowly over time through hydrolytic or enzymatic cleavage. One potential risk associated with polymer therapeutics is that extended clinical use of conjugates containing nondegradable or slowly degrading polymer fragments can lead to long-term vacuolization.[17,18] Therefore, the preparation of polycations that can degrade relatively quickly in the body to afford small molecules remains an important target.

This article reports the design and synthesis of a new class of pH-sensitive poly(amidoamine)s with enhanced degradability. A poly(amidoamine) backbone was chosen as it typically possesses many desirable properties, such as water solubility, lower toxicity compared to other cationic polymers, and synthetic flexibility as additional functionalities such as side-chain substituents can easily be incorporated.[19−24] These new poly(amidoamine)s were designed to remain stable at the physiological pH of 7.4 but degrade more quickly into many small molecules in the pH 5.0−6.0 environment of lysosomes, endosomes, or tumor tissues. A variety of acid-degradable bis(acrylamide) monomers have been prepared and allowed to react with piperazine via Michael addition to afford a series of pH-sensitive poly(amidoamine)s (Figure 1). These polymers contain acetals or ketals along the main chain for degradation by acid-catalyzed hydrolysis into low molecular weight compounds (Scheme 1). The rate of hydrolysis of these polymers could be tuned by varying the nature of the degradable linkage used in the acetal or ketal monomer. Another interesting family of acid degradable polymers containing ketal linkages has recently been reported by Heffernan and Murthy.[25]

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Experimental Section

General Procedures and Materials. All reagents were purchased from chemical suppliers and used without further purification unless otherwise noted. *p*-Toluenesulfonic acid was dehydrated and then recrystallized from toluene. *N*-(2-Hydroxyethyl)-(2,2,2-trifluoroacetamide) and acryloyl chloride were freshly distilled before use. After extractive workup, organic layers were combined and dried with anhydrous $MgSO_4$. To avoid hydrolysis of acid-sensitive compounds during chromatography separation on Merck Kieselgel 60 silica gel (230−400 mesh), 1% triethylamine was added to the solvents used for elution. NMR chemical shifts are reported in ppm relative to residual solvent peaks, and all coupling constants are reported in hertz. High-resolution fast bombardment mass spectrometry (FAB-HRMS) experiments were performed at the UC Berkeley mass spectroscopy facility. Fourier transform spectroscopy (FT-IR) was done using a thin film cast from CH_2Cl_2 on a reflective mirror surface. Elemental analyses were performed at the UC Berkeley analytical facility. Size exclusion chromatography was carried out on a Waters 410 (refractive index) apparatus with two columns in a series TSK-GEL (2500 PWxl) + TSK-GEL (3000 PWxl) using a 0.1 M Tris/0.1 M LiBr (pH 8.0) solution in distilled water as the eluent and poly(ethylene glycol) calibration standards.

Compound 1. *N*-(2-Hydroxyethyl)phthalimide (5.0 g, 2 equiv, 26 mmol) was added to benzene (200 mL). Then a small portion of benzene (20 mL) was removed by distillation at 95 °C using a short path distillation head while stirring to remove water from the system. After cooling the flask to room temperature (rt), acetaldehyde dimethylacetal (1.2 g, 1 equiv, 13 mmol) and *p*-toluenesulfonic acid (55 mg, 0.02 equiv, 0.32 mmol) were added. Benzene was then again removed by distillation in order to drive the reaction forward by removing methanol. Once distillation was complete, triethylamine (1 mL) was added to quench the acid. The product was purified by silica gel column chromatography using 4:1 hexane/ethyl acetate, 1:1 hexane/ethyl acetate, and finally ethyl acetate alone as the eluent. The product (4.8 g, 11.7 mmol, 38% yield) was obtained as a white solid; mp 133−134 °C. IR (cm^{-1}): 3469 (br, m), 1711 (s), 1394 (s). ^1H NMR (400 MHz, $CDCl_3$): δ 1.25 (d,

10.1021/ma062319v CCC: $37.00
Published on Web 01/12/2007

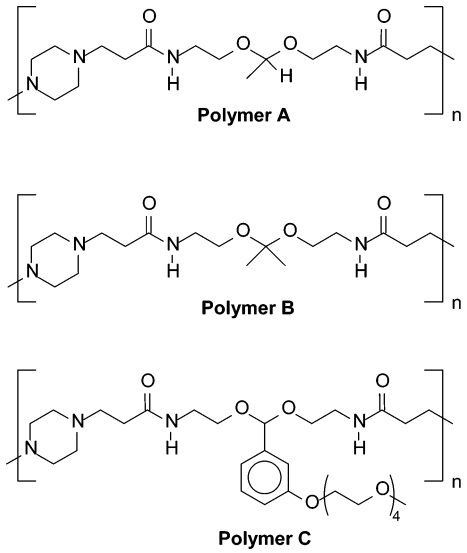


Figure 1. Acid-degradable poly(amidoamine)s.

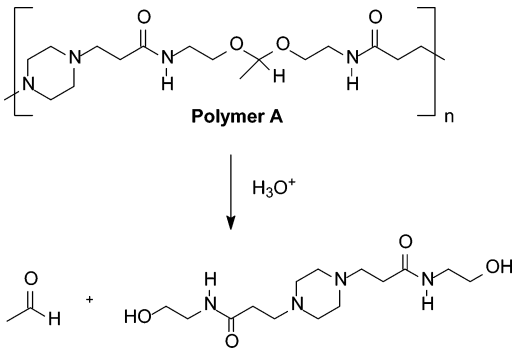
3H, $J = 5$), 3.36−3.92 (m, 8H), 4.78 (q, 1H, $J = 5$), 7.11 (m, 4H), 7.84 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 19.28, 37.71, 61.42, 99.03, 123.26, 132.06, 133.93, 168.17. Calcd: $[M + H]^+$ ($C_{22}H_{21}N_2O_6$) $m/z = 409.364$. Found: FAB-HRMS: $[M + H]^+ = 409.3651$. Anal. Calcd: C, 64.70; H, 4.94; N, 6.86. Found: C, 64.49; H, 4.91; N, 6.89.

Compound 2. *N*-(2-Hydroxyethyl)phthalimide (5.0 g, 2 equiv, 26 mmol) was added to benzene (100 mL). Then a small portion of benzene (20 mL) was removed by distillation at 95 °C using a short path distillation head while stirring to remove water from the system. After cooling the flask to rt, 2,2-dimethoxypropane (1.4 g, 1 equiv, 13 mmol) and *p*-toluenesulfonic acid (55 mg, 0.02 equiv, 0.32 mmol) were added. Benzene was then again removed by distillation in order to drive the reaction forward by removing methanol. Once distillation was complete, triethylamine (1 mL) was added to quench the acid. The reaction mixture was purified by column chromatography eluting with 1:1 hexanes/ethyl acetate to afford **2** as a white solid (2.3 g, 42%); mp = 150−153 °C. IR (cm^{-1}): 1765 (m), 1702 (s), 1390 (m), 1367 (m), 1016 (m). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.16 (s, 6H), 3.41 (t, 4H, $J = 6$), 3.62 (t, 4H, $J = 6$), 7.77 (m, 8). ^{13}C NMR (DMSO-d_6): δ 24.45, 37.58, 57.37, 99.93, 123.01, 131.41, 134.36, 167.61. Calcd: $[M + H]^+$ ($C_{23}H_{23}N_2O_6$) $m/z = 423.155$. Found: FAB-HRMS: $[M + H]^+$ $m/z = 423.156$. Anal. Calcd: C, 65.40; H, 5.25; N, 6.63 Found: C, 65.38; H, 5.36; N, 6.61.

Compound 3. Compound **1** (3.0 g, 7.3 mmol, 1 equiv) and 6 M NaOH (18 mL) were combined, and the reaction mixture was refluxed overnight at 105 °C followed by addition of dioxane (15 mL) after cooling. Upon complete removal of the acetamide groups, as determined by TLC using ninhydrin staining, the reaction mixture was cooled to 0 °C. Acryloyl chloride (13.4 g, 146 mmol, 20 equiv) and triethylamine (44.9 g, 438 mmol, 60 equiv) were added in small alternating portions with constant monitoring of pH. A 10% K_2CO_3 in water solution was added, the reaction mixture was stirred for 10 min, and the product was extracted with 6 × 100 mL portions of ethyl acetate. The product was purified by silica gel column chromatography using 1:1 hexane/ethyl acetate and finally with 100% ethyl acetate to afford **3** as a white solid (1.0 g, 55% yield); mp 70−71 °C. IR (cm^{-1}): 3429 (br, m), 1659 (s), 1556 (s), 1137 (s). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30 (d, 3H, $J = 5$), 3.45−3.69 (m, 8), 4.69 (q, 1H, $J = 5$), 5.65 (dd, 2H, $J = 10$, $J = 1$), 6.16 (dd, 2H, $J = 17$, $J = 10$), 6.22 (br, 2H), 6.30 (dd, 2H, $J = 17$, $J = 1$). ^{13}C NMR ($CDCl_3$): δ 19.49, 39.59, 63.79, 100.12, 126.43, 130.75, 166.04. Calcd: $[M + H]^+$ ($C_{12}H_{21}N_2O_4$) $m/z = 257.294$. Found: FAB-HRMS: $[M + H]^+ = 257.2942$. Anal. Calcd: C, 56.24; H, 7.87; N, 10.93. Found: C, 55.99; H, 7.98; N, 10.87.

Compound 4. Compound **2** (8.6 g, 0.02 mol, 1 equiv) and 6 M NaOH (100 mL) were combined, and the reaction mixture was

Scheme 1. Polymer Hydrolysis



refluxed overnight at 120 °C followed by addition of dioxane (50 mL) after cooling. Upon complete removal of the acetamide groups, as determined by TLC using ninhydrin staining, the reaction mixture was cooled to 0 °C. Acryloyl chloride (24.7 mL, 0.3 mol, 15 equiv) and triethylamine (127.5 mL, 0.9 mol, 45 equiv) were added in a small alternating portions with constant monitoring of pH. A 10% K_2CO_3 in water solution was added, the reaction mixture was stirred for 10 min, and the product was extracted into ethyl acetate. The organic layer was dried, and the crude mixture was purified by silica gel column chromatography with 1:1 hexane/ethyl acetate and finally with 100% ethyl acetate to afford **4** as a (3.4 g, 61% yield) white solid; mp 72−73 °C. IR (cm^{-1}): 3400 (br, m), 1657 (s), 1556 (s), 1059 (s). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30 (s, 6H), 3.53−3.58 (m, 8H), 5.69 (dd, 2H, $J = 10$, $J = 2$), 6.10 (br, 2H), 6.19 (dd, 2H, $J = 17$, $J = 10$), 6.34 (dd, 2H, $J = 17$, $J = 2$). ^{13}C NMR ($CDCl_3$): δ 24.74, 39.69, 59.11, 100.12, 126.58, 130.65, 166.08. Calcd: $[M + H]^+$ ($C_{13}H_{23}N_2O_4$) $m/z = 271.348$. Found: FAB-HRMS: $[M + H]^+ = 271.346$. Anal. Calcd: C, 57.76; H, 8.20; N, 10.36. Found: C, 56.89; H, 8.24; N, 10.34.

Compound 5. This compound was prepared according to a modified procedure reported by Loth et al.,[26] where tetraethylene glycol monomethyl ether was used instead of triethylene glycol monomethyl ether. Spectroscopic data corresponded to data reported in the literature.[3]

Compound 6. Chloride **5** (10 g, 44 mmol, 1.3 equiv) and *m*-hydroxybenzaldehyde (4.2 g, 34 mmol, 1 equiv) were dissolved in dry THF (20 mL). K_2CO_3 (4.6 g, 34 mmol, 1 equiv) was added followed by KI (40 mg, 0.24 mmol, 0.01 equiv) and 18-crown-6 (1.0 g, 3.7 mmol, 0.11 equiv). The reaction mixture was stirred at reflux for 48 h. The resulting mixture was cooled to rt, and water (100 mL) was added. The product was extracted with 4 × 150 mL portions of ethyl acetate, and the organic layers were combined and dried. The oil was loaded onto a silica gel column and eluted with 1:9, 1:4, 3:7, and 4:1 ethyl acetate/hexane and finally with ethyl acetate alone to afford **6** as a yellow oil with a minor amount of unknown impurity (8 g, 75%). IR (cm^{-1}): 2874 (s), 1697 (s), 1109 (s). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.36 (s, 3H), 3.52−3.76 (m, 17H), 3.87 (t, 2H, $J = 5$), 4.18 (t, 2H, $J = 5$), 7.18−7.22 (m, 1H), 7.39−7.46 (m, 3H), 9.95 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 42.60, 58.61, 67.39, 67.49, 69.31, 70.20, 70.32, 70.35, 70.55, 71.07, 71.67, 112.96, 121.57, 123.05, 129.88, 137.62, 159.20, 191.78. Calcd: $[M + H]+$ ($C_{16}H_{25}O_6$) $m/z = 313.1651$. Found: FAB-HRMS: $[M + H]^+ = 313.1632$. Anal. Calcd: C, 61.52; H, 7.74. Found: C, 60.98; H, 7.32.

Compound 7. Aldehyde **6** (2.0 g, 6.4 mmol, 1 equiv) and *N*-(2-hydroxyethyl)-2,2,2-trifluoroacetamide (7.4 g, 47 mmol, 7 equiv) were dissolved in dry THF (30 mL) followed by addition of *p*-toluenesulfonic acid (0.21 g, 1.2 mmol, 0.19 equiv) and 5 Å molecular sieves (30 g). The reaction mixture was stirred overnight and quenched with triethylamine the next day. Molecular sieves from the reaction mixture were removed by filtration. A 150 mL portion of water was added to the filtrate, and the reaction mixture was extracted with 5 × 150 mL portions of ethyl acetate. The ethyl acetate was evaporated, and the yellow oil was loaded onto the silica gel column. The product was purified by eluting the column with 2:1 hexane/ethyl acetate, 1:1 hexane/ ethyl acetate, 1:2 hexane/

ethyl acetate, and finally ethyl acetate alone to afford a light yellow solid. The product was recrystallized twice from ethyl acetate/hexane (1.9 g, 48% yield), although some starting aldehyde was still present as an impurity; mp 88−90 °C. IR (cm^{-1}): 3306 (b), 2886 (s), 1714 (s), 1564 (s), 1157 (s). ^1H NMR (300 MHz, $CDCl_3$): δ 3.34 (m, 5H), 3.49−3.86 (m, overlaps with solvent peak), 4.12 (br, 4H), 5.47 (s, 1H), 6.88−6.98 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 42.6, 55.9, 56.1, 60.6, 64.5, 68.3, 70.3, 72.1, 72.5, 73.5, 100.6, 113.8, 117.5 (q, $J = 275$), 119.0, 124.3, 128.2, 130.2, 132.5, 138.0, 156.7 (q, $J = 67$), 164.4. Calcd: $[M + H]^+$ ($C_{24}H_{35}O_9F_6N_2$) $m/z = 609.224$. Found: FAB-HRMS: $[M + H]^+ = 609.2246$. Anal. Calcd: C, 47.37; H, 5.63; N, 4.60. Found: C, 47.68; H, 5.32; N, 4.53.

Compound 8. Compound **7** (1.5 g, 2.5 mmol, 1 equiv) and 6 M NaOH (20 mL) were added to dioxane (10 mL), and the reaction mixture was stirred at rt for 4 h. Complete removal of the acetamide groups was determined by TLC using ninhydrin staining. Upon completion, the reaction mixture was cooled to 0 °C. Acryloyl chloride (1.3 g, 15 mmol, 6 equiv) and triethylamine (4.3 g, 44 mmol, 18 equiv) were added in small portions with constant monitoring of pH to maintain it above 7. A 10% K_2CO_3 in water solution was added, the reaction mixture was stirred for 10 min, and the product was extracted with 6 × 100 mL portions of ethyl acetate. The organic layer was dried, and the crude mixture was purified by silica gel column chromatography with 1:1 hexane/ethyl acetate and finally with 100% ethyl acetate, yielding product (0.8 g, 61%). IR (cm^{-1}): 3350 (br), 1659 (s), 1556 (s), 1102 (s). ^1H NMR (400 MHz, $CDCl_3$): δ 3.41 (s, 3H), 3.57−3.83 (m, 20H), 3.90 (t, 2H, $J = 5$), 4.17 (t, 2H, $J = 5$), 5.49 (s, 1H), 5.69 (dd. 2H, $J = 10$, $J = 2$), 6.20 (dd, 2H, $J = 17$, $J = 10$), 6.25 (br, 2H), 6.34 (dd, 2H, $J = 17$, $J = 2$), 6.93 (d, 1H, $J = 8$), 7.02−7.06 (m, 2H), 7.31 (m, overlaps with $CDCl_3$). ^{13}C NMR ($CDCl_3$): δ 39.21, 42.60, 58.31, 61.35, 64.44, 69.36, 70.34, 70.73, 71.09, 71.55, 100.68, 114.82, 117.86, 121.43, 123.06, 130.78, 131.63, 137.62, 159.20, 165.20. Calcd: $[M]^+$ ($C_{26}H_{40}O_9N_2$) $m/z = 524.2733$. Found: FAB-HRMS: $[M + H]^+ = 525.2812$. Anal. Calcd: C, 59.53; H, 7.69; N, 5.34. Found: C, 59.54; H, 7.89; N, 5.21.

Synthesis of Polymers A, B, and C. The Michael addition polymerization of degradable bis(acrylamide) monomers (compound **3** or **4**) with an equimolar piperazine was performed using water as a solvent. Compound **3** (1.0 g, 3.7 mmol) or compound **4** (0.95 g, 3.7 mmol) was reacted with piperazine (0.31 g, 3.7 mmol) in 2 mL of distilled water. Because of the partial solubility of monomer **8** (2.0 g, 3.8 mmol) in water, methanol was used as the solvent. To a stirred solution of bis(acrylamide) in water, piperazine was added, and nitrogen was flushed through the reaction mixture for 10 min. The temperature of the polymerization mixture was kept at 50 °C, and the reaction mixture was stirred for 7−8 h. The polymers were isolated by precipitation into acetone.

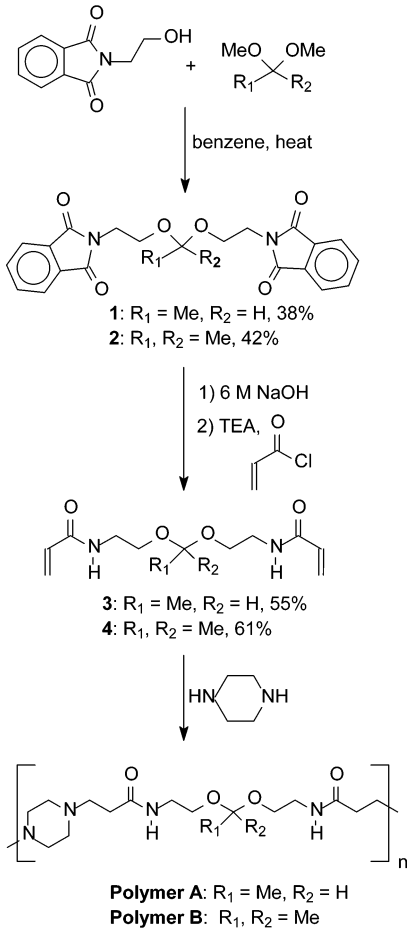
Characterization of Polymer A. IR (cm^{-1}): 3409 (br, m), 1644 (s). ^1H NMR (500 MHz, DMSO-d_6): δ 1.18 (d, 3H, $J = 5$), 2.19−2.49 (m, 18H), 3.17 (br, 4H), 3.48 (m, 2H) 4.65 (br, 1H), 8.03 (br, 2H). ^{13}C NMR (DMSO-d_6): δ 19.66, 33.75, 39.24, 52.86, 54.62, 64.28, 100.54, 171.84.

Characterization of Polymer B. IR (cm^{-1}): 3409 (br, m), 1640 (s). ^1H NMR (400 MHz, DMSO-d_6): δ 1.29 (s, 6H), 2.36 (br, 8H), 2.59 (br, 8H), 3.27 (br t, 4H, $J = 5$), 3.46 (br t, 4H, $J = 5$). ^{13}C NMR (DMSO-d_6): δ 24.5, 33.0, 39.5, 51.8, 53.3, 59.8, 101.2, 175.0.

Characterization of Polymer C. IR (cm^{-1}): 1394 (s), 1709 (s), 3469 (br, m). ^1H NMR (500 MHz, DMSO-d_6): δ 2.18−2.41 (m, 10H), 2.62 (br, 2H), 3.07−3.56 (m, overlaps with water peak), 3.71 (br, 2H), 4.05 (br, 2H), 5.47 (s, 1H) 6.89 (br, 1H), 6.97 (m, 2H), 7.25 (br, 1H), 8.05 (br, 2H). ^{13}C NMR (DMSO-d_6): δ 33.4, 33.7, 38.9, 45.6, 52.8, 54.2, 54.8, 58.4, 69.3, 70.0, 70.2, 70.3, 70.4, 71.6, 100.8, 113.7, 114.8, 119.8, 130.6, 140.8, 159.4, 171.2.

Hydrolysis of Polymers A, B, and C. A stock solution of deuterated phosphate buffer (D-PBS) was prepared by dissolving 0.03 M KCl, 0.02 M KH_2PO_4, 1.4 M NaCl, and 0.1 M $Na_2HPO_4 \cdot H_2O$ in deuterium oxide. Working D-PBS buffers with pH 5.0 and pH 7.4 were then prepared by adjusting the pH with 6 M NaOH. Hydrolysis experiments were started by adding 1 mL of pH 5.0 and 7.4 D-PBS solutions to 10 mg of the polymer with rapid stirring. The time $t = 0$ was taken as 1 min after mixing. Aliquots (0.5 mL) of each of the hydrolysis solutions were transferred to airtight sealed NMR tubes, and the spectra were taken at different time intervals. Samples were incubated in a 35 °C water bath in between spectra acquisition. 3-(Trimethylsilyl)propionic-*2,2,3,3-d*$_4$, sodium salt (δ −0.114), was used as an internal standard. The ratio (γ) of hydrolyzed polymer at time t was determined by comparing the disappearance of the integrated ^1H NMR spectra of the methyl protons (δ 1.18, d, 3H) or dimethyl protons (δ 1.27, s, 6H) or benzylidene proton (δ 5.49, s, 1H) for polymer A, B, and C, respectively, to the internal standard peak. The half-life of degradation was determined as $(\ln 2)/k_d$, where k_d was a negative value of the slope obtained by plotting $\ln(\gamma/\gamma_0)$ vs incubation time t. The reference ratio of hydrolyzed polymer to internal standard, γ_0, is the ratio at $t = 0$ (1 min after mixing).

Scheme 2. Preparation of Polymers A and B

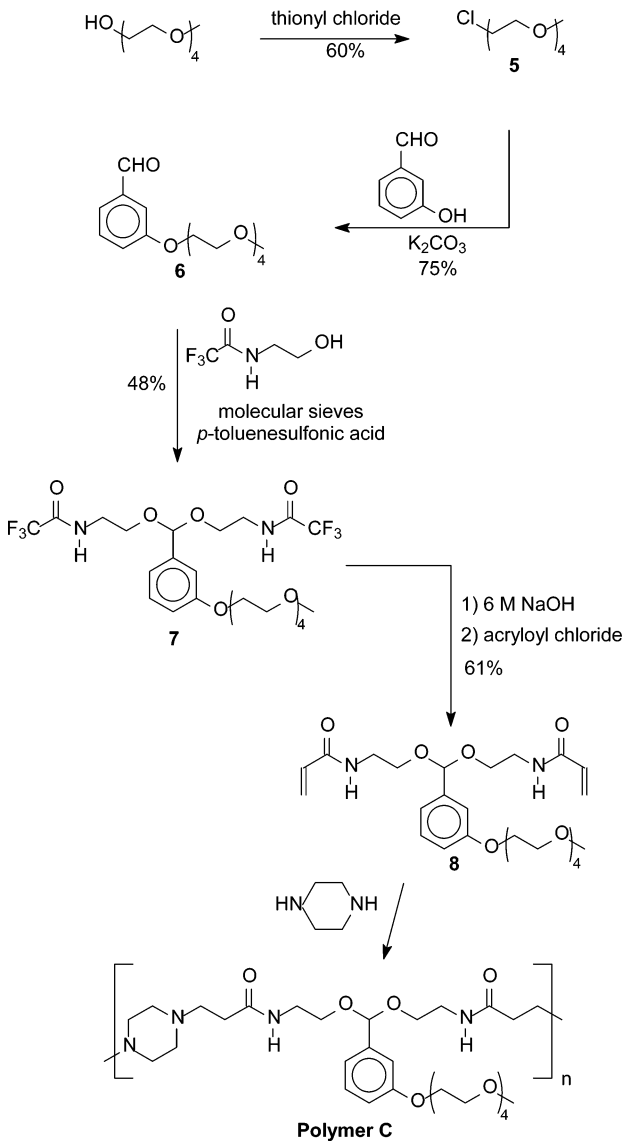


Results and Discussion

Design and Synthesis of Acid-Degradable, Bis(acrylamide) Monomers. Bis(acrylamide) monomers, **3** and **4**, containing acid-degradable acetaldehyde acetal and dimethyl ketal linkages, respectively, were synthesized in two steps (Scheme 2). *N*-(2-Hydroxyethyl)phthalimide was reacted with acetaldehyde dimethylacetal or 2,2-dimethoxypropane in the presence of a catalytic amount of acid in dry benzene using azeotropic distillation to drive the reaction forward. The intermediates were then deprotected with 6 M NaOH, and the products were reacted with an excess of acryloyl chloride to afford the desired monomers **3** and **4**.

Monomer **8**, based on a substituted benzaldehyde acetal, was synthesized in four steps as shown in Scheme 3. Compound **5** was synthesized according to a modified literature procedure[26]

Scheme 3. Preparation of Polymer C



and was then used to alkylate 3-hydroxybenzaldehyde using K_2CO_3 and 18-crown-6 to afford aldehyde **6**. Acetal **7** was prepared by reaction of aldehyde **6** with *N*-(2-hydroxyethyl)-2,2,2-trifluoroacetamide in the presence of a catalytic amount of *p*-toluenesulfonic acid. The final bis(acrylamide) monomer **8** was obtained by removal of the trifluoroacetamide protecting groups under strongly basic conditions followed by reaction of the resulting amines with an excess of acryloyl chloride.

Benzaldehyde acetals are well-suited for this application because their acid sensitivity can be tuned by the introduction of substituents at different positions of the aromatic ring. The introduction of an electron donating methoxy group at the *meta* position decreases the rate of acetal hydrolysis relative to the unsubstituted or *para* methoxy-substituted benzaldehyde derivatives.[27] In order to increase the solubility of the monomer in protic solvents, a hydrophilic oligoethylene oxide monomethyl ether substituent was attached onto the *meta* position of the aromatic ring of the aldehyde (Scheme 3). This modification not only decreased the rate of acetal hydrolysis of the benzaldehyde acetal but also improved the solubility of bis(acrylamide) monomer **8** in protic solvents.

Synthesis and Characterization of pH-Sensitive Poly(amidoamine)s. Poly(amidoamine)s are typically prepared by the stepwise polyaddition of primary or secondary amines to bis(acrylamide)s. Three poly(amidoamine)s were prepared containing different acid-degradable acetal or ketal linkages. The second-order hydrolysis rate constants of the linkages chosen for polymers A, B, and C varied from 0.248, 7.52×10^2, and 8.15, respectively, in 50:50 dioxane/water.[27,28] The polymerizations were carried out using an equimolar ratio of bis(acrylamide) monomers and piperazine in a protic solvent, either water or methanol, and the polymers were isolated by precipitation with acetone.[29−31] Aprotic solvents, even highly polar solvents, such as DMSO, were unsuitable as reaction media as they afforded only low molecular weight oligomers. The polymerization of degradable bis(acrylamide) monomers **3** and **4** with piperazine was performed using water as the solvent. Because of its partial solubility in water, polymerizations involving monomer **8** were performed in methanol. AA−BB type step-growth polymerizations require an equimolar ratio of monomers in order to yield high molecular weight materials; therefore, relatively large-scale reactions were conducted to avoid any weighing errors, and high-purity monomers were used to avoid a stoichiometric imbalance.

Table 1. Characterization of the Polymers[a]

				half-life (days)	
polymer	M_n (kDa)	M_w (kDa)	PDI	pH 5.0	pH 7.4
A	9.8	18.8	1.9	81	161
B	3.3	6.5	2.0	0.03	6
C	6.4	13.5	2.1	3	15

[a] SEC using poly(ethylene glycol) calibration standards.

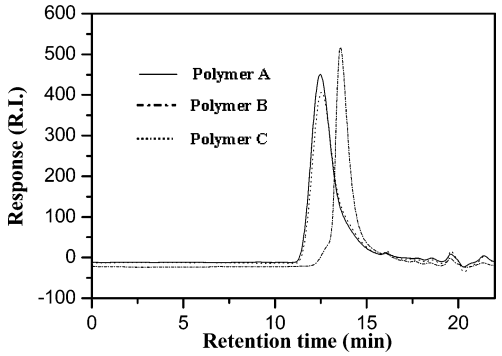


Figure 2. SEC traces of polymers A, B, and C.

The molecular weight of each polymer, as measured by size exclusion chromatography, is given in Table 1 and Figure 2. A slightly basic aqueous SEC setup was used to avoid degradation of the polymer during analysis, and the molecular weights were measured against poly(ethylene glycol) calibration standards. The M_w of the polymers varied from 6.5 to 18.8 kDa, and the PDIs for all the polymers ranged from 1.9 to 2.1 as expected for a step-growth polymerization process. The variation in the molecular weight of the polymers is probably due to the difference in the rate of hydrolysis of the acid-degradable linkages. As mentioned earlier, this polyaddition only proceeds well in protic solvents, which may also promote the degradation of the pH-sensitive linkages present within the polymer backbone. It is suspected that the acetals underwent hydrolysis, especially in the case of polymer B, which contains the most acid-sensitive linkage, leading to lower molecular weight structures. Degradation of the acetal during polymerization not only causes scission along the polymer backbone but may also lead to monomer degradation resulting in a stoichiometric imbalance. If a small amount of bis(acrylamide) monomer was hydrolyzed, then byproducts would be formed that could cap the ends of growing polymer chains. In a control experiment, the Michael addition polymerization was also performed with

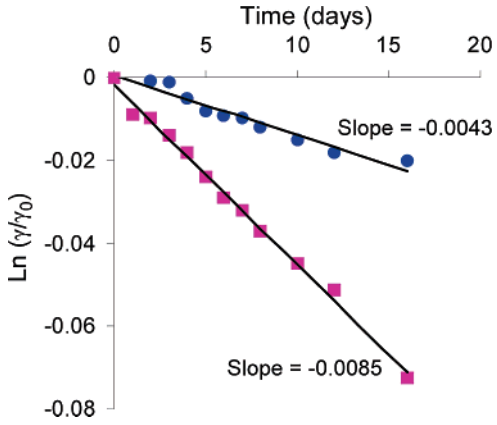


Figure 3. Hydrolysis kinetics of polymer A at pH 5.0 (■) and pH 7.4 (●).

nondegradable *N*,*N*′-methylenebis(acrylamide) and piperazine under the same conditions, leading to a product with a molecular weight of 52 kDa. This finding suggests that the lower molecular weights obtained with the degradable bis(acrylamide) monomers are likely due to hydrolysis of the acid-degradable linkages. Although only relatively low molecular weight poly(amidoamine)s were obtained, they should still be very useful for the delivery of biotherapeutics because larger cationic macromolecules induce higher cytotoxicity than their lower molecular weight counterparts.[32−34]

Effect of pH on the Degradation Kinetics of Poly(amidoamine)s. The hydrolysis kinetics of the polymers were measured at pH values corresponding to those typically found in lysosomes (pH 5.0) and in the blood (pH 7.4) at 35 °C. The hydrolysis rate constants and half-lives of the polymers were calculated by monitoring the disappearance of polymer peaks via NMR spectroscopy and using the Arrhenius equation (i.e., first-order decay).[35]

Hydrolysis half-lives calculated for polymers A, B, and C are given in Figures 3−5 and Table 1. As expected, all polymers degraded faster in mildly acidic environments than at physiological pH. The acceleration of the hydrolysis kinetics of the polymer from pH 7.4 to pH 5.0 is expected since the rate of acetal hydrolysis is proportional to the hydronium ion concentration. At both pH 5.0 and pH 7.4, the rate of degradation of the polymers was in the order B > C > A. At pH 5.0, the half-life of polymer B was 100 times shorter than that of polymer C, which itself had a half-life 27 times shorter than that of polymer A. Similarly at pH 7.4, polymer B degrades much faster than polymers A and C. The difference in the rate of degradation of A, B, and C is related to the difference in the rate of hydrolysis of the acetal linkages present within the backbones of the polymeric chains.[36] The degradation of polymer B was also compared to the degradation of a small neutral molecule found in the literature that contained the same dimethyl ketal linkage.[35] The hydrolysis kinetics of the small molecule were previously measured using a similar NMR technique in the same pH 5.0 and pH 7.4 solvent systems as polymer B.[35] Degradation of polymer B was slower most likely due to the local buffering environment provided by the protonated tertiary amines. This behavior would also be expected for polymers A and C.

Conclusion

A family of acid-degradable poly(amidoamine)s that hydrolyze more quickly in mildly acidic conditions than at physiological pH has been prepared. This new class of polycationic macromolecules should prove useful as materials for drug

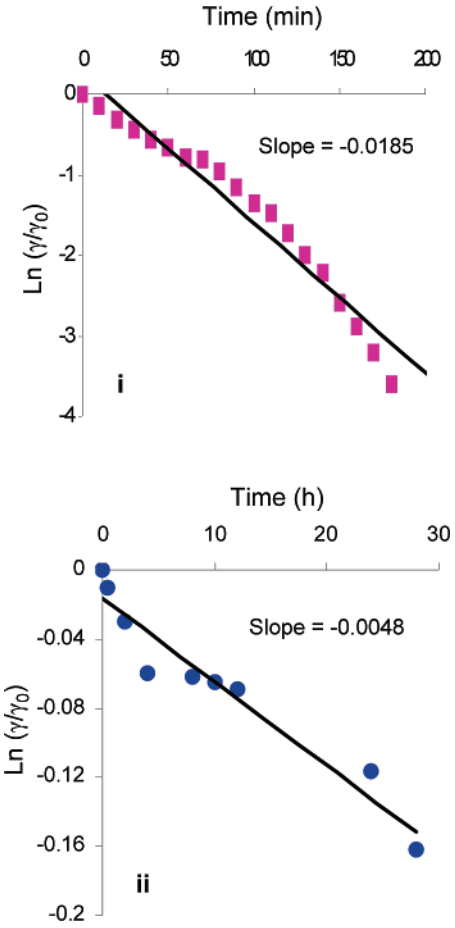


Figure 4. Hydrolysis kinetics of polymer B at (i) pH 5.0 and (ii) pH 7.4.

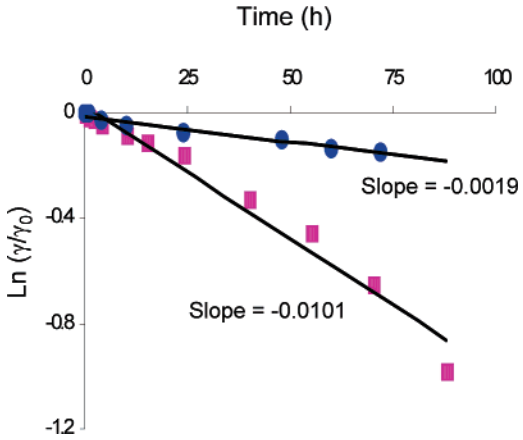


Figure 5. Hydrolysis kinetics at polymer C at pH 5.0 (■) and pH 7.4 (●).

delivery because of the ability to tune the pH sensitivity and the rate of polymer degradation. On the basis of these preliminary results, drug delivery systems prepared with the new acid-degradable poly(amidoamine)s may help modulate the release of therapeutic agents. The improved degradability should not only prevent vacuolization of the polymer but may also assist in the dissociation of biotherapeutics from the polymer scaffold[37,38] or facilitate endosomal escape due to membrane destabilization induced by the hydrolysis of the polymers into many small molecules.[4,39] We are currently evaluating the toxicity of this family of polymers as well as their byproducts to assess their applicability in a variety of polymer therapeutic applications.

Acknowledgment. Financial support of this research by the National Institutes of Health (grant 1RO1-EB005824 and PEN grant HL080729) is acknowledged with thanks. We also thank Dr. Marian Snauko for assistance with size exclusion chromatography studies.

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MA062319V